Association between chronic stress and Alzheimer effects of Saffron

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ABSTRACT

Chronic stress and high levels of glucocorticoids produce functional and structural changes in brain and especially in the hippocampus, an important limbic system structure that plays a key role in cognitive functions including learning and memory. Alzheimer's disease (AD) is a chronic neurodegenerative disease that usually starts slowly and worsens over time. Indeed, cognitive dysfunction, neuronal atrophy, and synaptic loss are associated with both AD and chronic stress. Recent preclinical and clinical studies have highlighted a possible link between chronic stress, cognitive decline and the development of AD. It is suggested that Tau protein is an essential mediator of the neurodegenerative effects of stress and glucocorticoids towards the development of AD pathology. Recent findings from animal and humans studies demonstrated that saffron and its main constitutive crocin are effective against chronic stress-induced cognitive dysfunction and oxidative stress and slowed cognitive decline in AD. The inhibitory actions on acetylcholinesterase activity, aggregation of beta-amyloid protein into amyloid plaques and tau protein into neurofibrillary tangles, and also the antioxidant, anti- inflammatory, and the promotion of synaptic plasticity effects are among the possible mechanisms to explain the neuroprotective effects of saffron. New evidences demonstrate that saffron and its main component crocin might be a promising target for cognition improvement in AD and stress-related disorders.

1. Alzheimer's disease

Alzheimer's disease (AD) is the most common cause of dementia in elderly people [1]. AD is an insidious disease, which occurs generally in people over the age of 55 and worsens with age. Population-based studies show that the age-specific incidence and prevalence of Alzheimer's dementia increase markedly after the age of 65 [2,3]. The clinical period is characterized by a gradual decline in intellectual and mental functioning, inability to perform daily activities, and ongoing changes in personality and behavior [1,4]. AD is accompanied by symptoms such as progressive, gradual, and irreversible dementia, memory loss, behavioral changes such as pessimism, loss of social status, and decreased speech without altering sensorimotor functions [5–7].

The molecular mechanisms of AD are very complex. The key events leading to AD appear to be the formation of numerous amyloid plaques, which clusters into amyloid plaques, or senile plaque on blood vessels and the outside surface of neurons of the brain, and intracellular aggregation of microtubule-associated Tau proteins as neurofibrillary tangles. The major component of amyloid plaques is amyloid-beta (Aß), results from the amyloid precursor protein (APP), which is present in the brain and peripheral tissues [8–10]. The presence of Aβ and its

Abbreviations: AD, Alzheimer's disease; APP, amyloid precursor protein; Aβ, amyloid beta; HPA, hypothalamic-pituitary-adrenal; ACTH, adrenocorticotropic hormone; GC, glucocorticoid; ROS, reactive oxygen species; NMDA, N-methyl-D-aspartate receptor; LTP, long-term potentiation; SOD, *Superoxide dismutase*; GPx, glutathione peroxidase; GSH, glutathione; MDA, malondialdehyde; TBARS, thiobarbituric acid reactive substances; AChE, acetylcholinesterase.

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plaques in brain cortical areas is the main hallmark of AD. Tau proteins are a group of structural proteins with physiological functions, especially in microtubules, but in the case of hyperphosphorylation, they become toxic insoluble aggregated proteins involved in pathophysiology of AD. Tau hyperphosphorylation can lead to abnormal folding, fragmentation, aggregation and/or the development of deposits known as neurofibrillary tangles [11]. The Tau hypothesis suggests that Tau tangle pathology precedes Aβ plaque formation and that Tau phosphorylation and aggregation is the primary cause of neurodegeneration in AD [12]. These extracellular plaques and intracellular tangles damage the healthy brain cells around them, leading to the death of damaged cells and shrinking the brain. These changes cause the symptoms of AD, such as memory loss, speech problems, confusion, and mood swings. Although the underlying mechanisms leading to the development of AD are not clearly defined, many risk factors have identified including age, genetic predisposition, exposure to the metals, traumatic brain injury, lifestyle, malnutrition, diabetes, immune system dysfunction, vascular disease, infectious agents, and psychiatric factors [13,14] (Fig. 1). Some possible causes are chronic stress, oxidative stress, and inflammation. Recent evidence support the etiopathogenic roles of chronic stress and glucocorticoids (GCs), oxidative stress, and chronic inflammation in the development and manifestation of AD [15–18]

2. Chronic stress and development of AD

Sufficient evidences from experimental and human studies have shown that chronic stress impairs cognitive functions in many ways. Animal studies showed that chronic stress and high levels of GCs cause structural remodeling in the brain structures including the prefrontal cortex and the hippocampus such as dendritic shortening and spine loss [19,20], neuronal atrophy [21], and the inhibition of neurogenesis [22], that result in cognitive impairments [23,24]. Oxidative stress can be one of the mechanisms by which chronic stress or GCs negatively affect learning and memory [25] and cause neurological damage [26–28]. Oxidative stress may increase with the augmented production of reactive oxygen species (ROS) or with decreased antioxidant enzymes [29]. In humans, chronic stress during early life or adulthood also has an impact on the recognition and the development of different psychopathologies, although individual differences are showing that various factors such as sex and genetic makeup may contribute in the development of different stress-related mental health diseases such as mild cognitive impairment and AD [30].

The hypothalamic-pituitary-adrenal (HPA) axis activation occurs as a biological response to stress. It leads to elevated plasma levels of adrenocorticotropic hormone (ACTH) and corticosteroids. In subjects who are under chronic stress, depending on the sensitivity, type, frequency, and severity of the stressor, various changes occur in the HPA

axis function including chronic basal hypersecretion, sensitized stress responses, or adrenal exhaustion [31]. The HPA axis function changes have been shown in elderly patients with anxiety disorders, patients with posttraumatic stress disorder, and subjects with high levels of neurotoxicity [32–34]. Accordingly, studies on humans showed that high levels of corticosteroids might enhance the progression and the risk of AD. In a sample consisted of 1865 cognitively healthy subjects, elevated urinary cortisol levels could predict the increment in the risk of AD by an average of 6 years before the onset of the disease [35]. In fact, many AD patients hypersecrete GCs, and their levels of GCs correlate with the rate of cognitive impairment and extent of neuronal atrophy [36]. Increased cortisol levels in patients with mild cognitive impairment and patients with dementia were also related to rapid cognitive decline [37,38]. In the meantime, these studies are still inconclusive because several other studies are now in progress that indicate a relationship between increased cortisol levels and AD progression [39–41].

Evidence from animal models indicate that exposure to stress and elevated cortisol levels may straightly increase the pathological processes of AD. In a rodent model of AD, Green et al. (2006) showed that treatment with dexamethasone results in enhanced pre-amyloid protein levels and the formation of cerebral amyloid plaques [42]. Treatment with dexamethasone also reduces brain β-amyloid secretion [43]. In wild rodents, chronic stress and GC treatment-induced Tau hyperphosphorylation, which is the first step in the creation of neurofibrillary tangles [44]. In fact, it is suggested that Tau is an essential mediator of the neurodegenerative effects of stress and GCs towards the development of AD pathology [15]. As aforementioned, Tau is a microtubule-associated protein that is stored in neuronal axons in physiological conditions. In AD, Tau sorting mechanisms fail and Tau

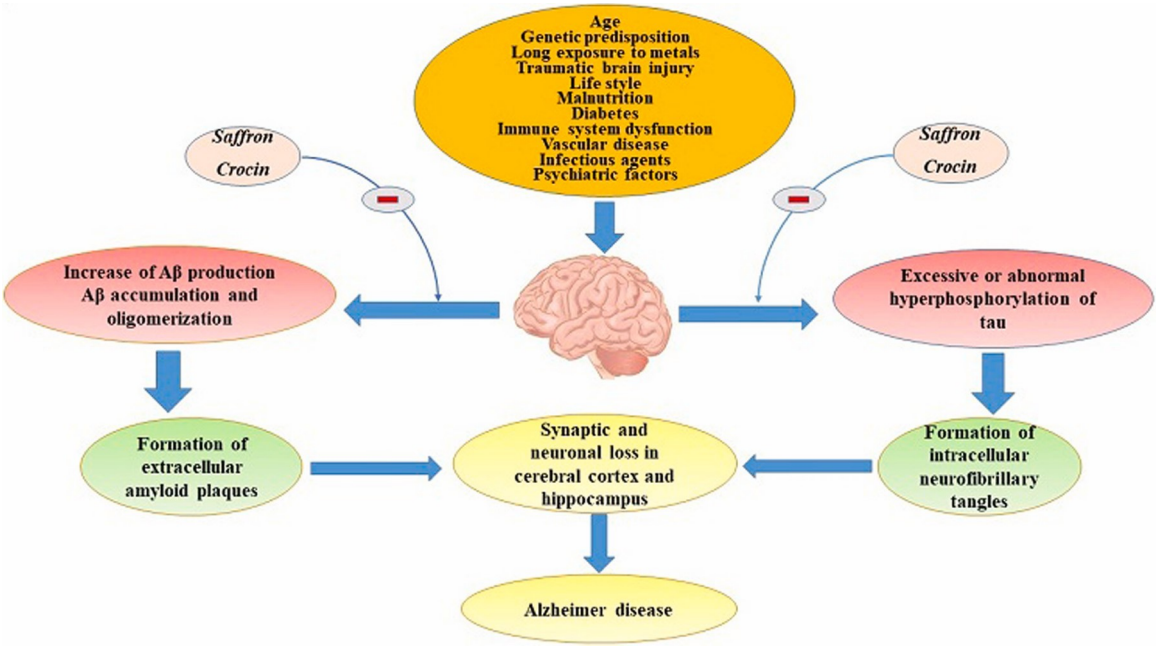

Fig. 1. Proposed pathways of Alzheimer's disease (AD) promotion by chronic stress, and therapeutic effects of saffron. Several important risk factors for AD age, genetic predisposition, long exposure to metals, traumatic brain injury, life style, malnutrition, diabetes, immune system dysfunction, vascular disease, infectious agents, and psychiatric. Chronic stress exacerbates abnormal Tau hyperphosphorylation and intracellular neurofibrillary tangles. Chronic stress also enhances the formation of amyloid beta and extracellular amyloid plaques. These extracellular plaques and intracellular tangles damage the healthy brain cells around them, leading to synaptic and neuronal loss in brain structures such as hippocampus and cerebral cortex, leading to AD. Saffron and its main constitute crocin have therapeutic effects against AD via decreasing Tau hyperphosphorylation and amyloid beta formation.

becomes missorted into the somatodendritic compartment, which might trigger by aberrant Aβ production [45]. Tau missorting in neurons causes degeneration of synapses, dendritic remodeling, and spine atrophy, which leads to impairments of neuronal functions. The findings showed that there is a 20− 30 year interval between the first abnormal changes in brain amyloid biomarkers and the onset of clinical symptoms [46,47]. This step of the illness, where the pathological processes are in progress in the brain while there are no clinical symptoms, is known as the preclinical AD [48].

Experimental evidences indicate that chronic stress and GCs have a dual role in AD development. They can directly trigger Tau hyper-phosphorylation in the hippocampus and prefrontal cortex of healthy, wild type, middle-aged rats, suggesting their primary role in the development of AD [42,44]. Moreover, exogenous GCs potentiate the ability of centrally infused Aβ to induce hyperphosphorylation of Tau epitopes associated with AD and cytoplasmic accumulation of Tau, and prior exposure to stress aggravates the biochemical and behavioral effects of GCs in Aβ-infused animals [42,44]. Thus, lifetime stress/GC exposure may have a cumulative impact on the onset and progress of AD pathology, with Tauhyperphosphorylation serving to transduce the negative effects of stress and GCs on cognition [44]. In agreement with this hypothesis, animals studies have shown that chronic psychological stress intensified cognitive deficits, accentuated the disruption of signaling molecules levels, and produced a greater depression of long-term potentiation (LTP) [49] by a mechanism involving decreased CaMKII activation along with increased calcineurin levels in Aβ rat model of AD [50].

The vicious cycle of stress model presented by Justice (2018) may explain the relationship between stress and AD [51]. According to this model (Fig. 2), on the right arc of the cycle, chronic stress and thus, elevated levels of GCs exacerbate AD, lead to the more rapid development of neuronal pathology and loss in cognitive function. On the left side of the cycle, AD disturbs stress response neural networks, making neuropsychiatric co-morbidities including depression, anxiety, and aggressive behavior. The HPA axis has a central role in both the exacerbation of AD by stress and the stress-related symptoms caused by on-going neurodegeneration [51].

3. Oxidative stress and development of AD

There is considerable evidence about the effects of oxidative stress on the brain as well as its association with AD, which shows that brain tissue in patients with AD is subjected to oxidative stress [17,18]. Activation of the HPA axis may also cause oxidative stress, thereby leading to synaptic dysfunction and neuronal apoptosis [52–54]. Since oxidative stress is determined by an imbalance in the radical production of ROS and antioxidant defense, both of them seem to play important roles in age-related neurodegeneration and cognition impairment [55–63]. Evidence of oxidative stress in patients with AD is obtained via high amounts of oxidized proteins, advanced glycation end products, lipid peroxidation end

products, the formation of toxic types such as peroxides, alcohols, aldehydes, free carbonyls, ketones, and specific oxidative changes in the mitochondrial and nucleus DNA [64–75]. Age -related memory impairments are associated with decreased defense mechanisms of brain and plasma antioxidants [76,77]. A substantial part of the antioxidant defense system is the low molecular weight thiol compound glutathione (GSH), which is responsible for the endogenous redox potential of the cell [78]. The most significant function of glutathione is to donate electrons to ROS and to protect them thereby. In various animal models, the intracellular GSH concentration declines with age [79–84]. It diminishes in various brain regions of the mammals, such as the hippocampus [85–87]. GSH depletion leads to a condition in which, the ROS production increases beyond the antioxidant capacity, which results in oxidative stress. Another reason for the oxidative stress is the imbalance in radical detoxification enzymes in AD [88]. Several researches have presented evidence of the adverse results of oxidative stress products on specific cellular targets in AD. Mitochondrial DNA oxidation has been observed in the parietal cortex of patients with AD and also in elderly people without AD [89], however less than nucleus DNA. Also, protein oxidation has been observed in elderly people with and without AD; however, it may be of much more prominence in areas with the most severe histopathologic variations in AD patients [90]. Many studies have shown that lipid peroxidation is enhanced in the brain of patients with AD, especially in the temporal lobe, where histopathological changes are highly significant [91–93].

4. Chronic inflammation and development of AD

Chronic inflammation is one of the major agents in the pathophysiology of AD [16]. Chronic neuro-inflammation alters adult neurogenesis: the generation of new neurons throughout adulthood in the

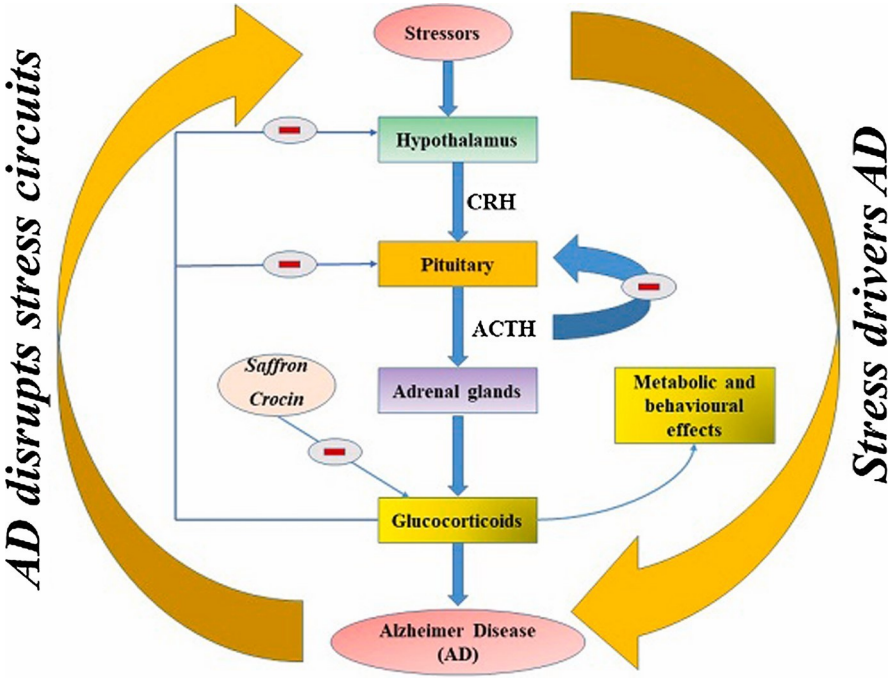

Fig. 2. The vicious cycle of stress proposed by Justice (2018). Stressors aggravate Alzheimer's disease (AD), leading more rapid development of pathology and impairment of cognitive function (right side). AD disturbs stress responsive neural circuits, producing neuropsychiatric disorders such as depression, anxiety, and aggressive behavior (Left side). The hypothalamic–pituitary–adrenal axis (center) is a complex set of direct influences and feedback interactions among three components: Hypothalamus, pituitary and adrenal gland. Release of corticotropin-releasing hormone (CRH) from the hypothalamus is stimulated by stress stimuli. CRH, in turn, activates pituitary ACTH release and subsequent glucocorticoids (GCs) release by the adrenal cortex. In addition to many metabolic and behavioral effects of GCs, elevated GCS levels have a fundamental role in both stress – associated neuropsychiatric disorders and the exacerbation of AD by stress. Saffron and crocin can reduce GCs levels.

sub-ventricular zone and the dentate gyrus. Most studies on β-amyloid in AD models have shown that following the induction of AD, the neurogenesis rate decreases significantly in both the ventricular and the dentate gyrus areas [94,95]. The presence of various inflammatory components, such as immune cells, cytokines, or chemokines, plays a role in regulating the survival, proliferation, and maturation of neural stem cells. Increased numbers of active microglia, astrocytes, cytokines, and reactive oxygen create an inflammatory response in this disease, which finally leads to loss of neurons and synapses in the cerebral cortex [96,97].

5. Current drugs for treatment of AD

Currently, two types of drugs are used to treat cognitive symptoms: AChE inhibitors including donepezil, rivastigmine, galantamine, tacrine [98], and the N-methyl-D-aspartate receptor (NMDA) antagonist memantine [99]. The first group drugs inhibit the breakdown of acetylcholine, an important brain neurotransmitter involved in cognitive functions. As AD progresses, the brain produces less and less acetylcholine; therefore, cholinesterase inhibitors increase the bioavailability of acetylcholine in the brain, and compensate for the loss of acetylcholine. Acetylcholinesterase

inhibitors are prescribed for mild to moderate AD [100]. The NMDA receptor antagonist memantine blocks the NMDA receptor of the glutamate neurotransmitter. Excitatory glutamatergic neurotransmission via the NMDA receptor is critical for synaptic plasticity and survival of neurons. However, excessive NMDA receptor activity causes excitotoxicity and promotes cell death, underlying a potential mechanism of neurodegeneration occurred in AD. The NMDA receptor antagonist is prescribed to treat moderate to severe AD [101]. Since NMDA antagonists work differently from cholinesterase inhibitors, combination of the two types of drugs are currently preferred therapies for AD.

Since the current drugs for the treatment of AD have side effects and are not completely effective, there is a serious need to find new treatments and new medications. The use of herbal medicine can be a new and complementary treatment. Herbal remedies are used by about 80 % of the population primarily in developing countries for primary health care [102,103]. Some important herbal plants for brain health and memory are *Ginkgo biloba* (family: Ginkgoaceae) *Huperzia Serrata* (family: Lycopodiaceae), *Salvia officinalis* (family Labiatae/Lamiaceae), *Melissa officinalis* (family: *Lamiaceae*) and *Crocus Sativus L* (saffron) (family: Iridaceae). Among these plants, recently saffron and its main constituent crocin have considered for treatment of neurodegenerative diseases including AD in human and animal models. In the following sections, we will review the effects of saffron and crocin on learning and memory, stress and glucocorticoid pathways, brain oxidative damage and neurotoxicity, and treatment of AD.

6. Saffron (Crocus Satious L, Iridaceae): the composition, effects on learning and memory, stress-related pathways, and neuroprotection

6.1. Saffron and its constituents

Saffron is the most expensive traditional spice and the priciest herb around the globe. Dried saffron has long been used as the food seasoning [104–106]. Saffron is a small, perennial plant that belongs to the iris family and is bulbiferous with a spherical bulb covered with thin brown membranes. Some narrow and elongated leaves grow out from the bulb or the stem base. From the leaves, the pedicles grow, giving one to three flowers. Flowers include 3 stamens and a pistil leading to the red to orange stigma. The usable part is the terminal part of the style and stigma known as saffron, with an aromatic scent and a slightly bitter flavor. The total annual production of saffron is about 205 t, more than 80 % of which is harvested in Iran [107]. Saffron is planted in the Mediterranean countries such as Iran, Spain, France, and Greece to China and India. Geographical distribution of saffron in Iran includes Khorasan, Yazd, Kerman, Gilan, and Mazandaran provinces [108]. Saffron is not only a widely applied food seasoning; but also it has numerous pharmacological effects. Oral consumption of saffron in small amounts (a daily dose of 100 mg of saffron or 30 mg of saffron hydroalcoholic extract)

may induce significant pharmacological effects [109–114]. One of the main ingredients of saffron is crocin, which accounts for the yellow pigmentation of the stigmas. Another important component is picrocrocin, which gives the rusty, bittersweet flavor. Safranal is another important organic substance of saffron, which is primarily responsible for the aroma of saffron. Other carotenoids such as beta-carotene, lycopene, and xanthine and vitamins, especially riboflavin and thiamin, are also found in saffron [115]. Chemical analysis of pigments in the saffron indicates the presence of crocin as a water-soluble carotenoid, aldehyde monoterpene, and glucoside (safranal and picrocrocin) and flavonoids (quercetin and kaempferol) [116].

Saffron is part of a long-lasting tradition in history of medicine. In traditional medicine, saffron is used as an antispasmodic, gum sedative, nerve sedative, appetizer, aphrodisiac, and emmenagogue agent [117]. Recent studies indicated that saffron has anti-carcinogenic, anti-mutagenic [118], and immune-modulating effects. These effects are mainly due to antioxidant agents in the saffron such as volatiles (e.g., safranal), bitter agents (e.g., picrocrocin), and pigments (e.g. crocin) [117]. Saffron and crocin both induce free radical disinfectant and antioxidant activities. Besides, these compounds also protect the DNA of mice against genotoxin-related oxidative stress and methyl methane sulfate-linked damages [119]. Saffron essential oil has protective role against genotoxin-induced oxidative stress in Swiss albino mice [120]. Moreover, new pharmacological studies have shown that saffron essential oil or its active compounds have anticonvulsant [121], anti-depressant [122], anti-inflammatory [123] and radical scavenger and learning and memory improvement properties [117,124–127] and promote the release of oxygen in various organs [117]. Saffron essential oil also shows gene protection effects and protects against oxidative stress-induced by genotoxins in mice [120,128–130].

6.2. Saffron, learning, and memory

Animal studies have revealed that saffron and crocin have cognitive improving effects in a wide variety of conditions. In *in vitro* environment, crocin has a weak but remarkable affinity for the NMDA receptor [131], and it prevents ethanol inhibition of the NMDA receptor in rat hippocampal neurons [132]. The NMDA receptor is an ion channel receptor found at most excitatory synapses and gated by the excitatory neurotransmitter glutamate. This receptor plays an important role in LTP in brain structures such as the hippocampus. After high-frequency stimulation of presynaptic neurons, the hippocampal excitatory synapses show a long-term increase in synaptic potentials. This phenomenon is called LTP, which is a type of synaptic plasticity and involves a part of the cellular basis of learning and memory [133]. Ethanol extract of *Crocus sativus L.* antagonizes ethanol or acetaldehyde-induced inhibition of hippocampal LTP in rats, suggesting that saffron can prevent aversive effects induced by ethanol and its metabolite acetaldehyde [127,134]. A single oral administration of an

alcohol extract of saffron reduced the ethanol induced passive avoidance memory impairment, a form of associative learning and memory in rodents [126]. Saffron and its active agents, i.e. crocin and safranal, also have no effect on intact memory, but can prevent scopolamine – induced spatial memory loss [135]. In rats, post-training administration of saffron (30 and 60 g/kg) successfully counteracted the extinction of recognition memory in the normal rat, suggesting that saffron modulates storage and/or retrieval of information. Pre-training treatment of the same doses of saffron significantly antagonized the scopolamine induced performance deficits in the step-through passive avoidance test [136]. Crocin at a dose of 30 mg/kg can antagonize the streptozocin (STZ)-induced model of sporadic AD in male rats [137]. In a recent work, we have shown that saffron and its active ingredient crocin can prevent the impairment of learning and memory as well as the oxidative stress damage to the hippocampus induced by chronic stress [138]. We also recently reported that crocin treatment can overcome behavioral deficits induced by adolescent stress in rats [139]. These findings together demonstrate that saffron and its active ingredients may be useful in pharmacological alleviation of cognitive deficits seen in neurodegenerative disorders such as AD. *6.3. Links between saffron effects and stress or glucocorticoid pathways*

Corticotrophin-releasing hormone (CRH) is a 41-amino acid peptide, which is released by the paraventricular nucleus of the hypothalamus in response to stress stimuli. CRH also is released in the hippocampus and amygdala and regulates behavioral and autonomic responses to the stress [140]. It is reported that crocin can reduce the CRH expression in stressed rats and thus reduce the HPA activity and stress effects [141]. Saffron and crocin can prevent chronic stress-induced (21 days) learning and memory impairment, oxidative damage of the brain, liver, and kidney and reduce glucocorticoid levels in rats, showing their inhibitory effects on the HPA axis [138,142]. It is also reported that systemic administration of saffron water extract could abolish stress-induced corticosterone plasma level, and metabolic and behavioral symptoms of acute stress in male rats [143]. Pretreatment systemic administration of saffron aqueous extract or crocin could prevent stress-induced enhancement of plasma corticosterone and metabolic and behavioral response in mice, suggesting an interaction between saffron aqueous extract or crocin with the HPA axis for reduction of the stress-induced corticosterone release [144]. These findings together suggest that the downregulation of the HPA activity by saffron or crocin is one of the important mechanisms that underline the protective role of these agents against stress-related disorders.

6.4. Saffron and oxidative damages and neurotoxicity

It is reported that an injection of crocin (100 mg/kg) for a week can attenuate neurobehavioral and neurochemical changes most probably by its antioxidant property in cerebral ischemia in rats [145]. A study has shown neuroprotective effect of saffron extract (5 and 25

mg/mL), and crocin (10 and 50 μM) in glucose-induced neurotoxicity using PC12 cells as a suitable *in vitro* model of diabetic neuropathy, suggesting that saffron and its carotenoid crocin could be potentially useful in diabetic neuropathy treatment [146]. An injection of aluminum chloride ($AlCl_3$) for 45 days induced alterations in some biochemical parameters inducing oxidative damage representing by increase in TBARS and inhibiting the activities of antioxidant enzymes such as superoxide dismutase (SOD), and plasma glutathione peroxidase (GSH-Px) in the brains of Balb/c and C57BL/6 mice. Co-administration of aqueous saffron extract (200 mg/kg) with $AlCl_3$ decreased its hazards [147]. A study has reported that safranal (72.75–727.5, mg/kg, i.p), an ingredient of saffron, can attenuate cerebral ischemia induced oxidative damage in rat hippocampus in a dose-dependent manner [148,149]. Safranal also decreased the extracellular concentration of glutamate and aspartate in the hippocampus of anesthetized rats following the administration of kainic acid [150]. Besides, crocin increased SOD and glutathione peroxidase (GPx) activity and made a significant decrease in malondialdehyde (MDA) in ischemic stroke in rat models [151]. Simultaneous consumption of saffron essential oil with aluminum reversed changes in monoamine oxidase activity that has been associated with the amount of lipid peroxidation in the whole brain and cerebellum [152]. It has been proposed that exposure to excessive amounts of GCs or chronic stress may lead to oxidative damage to the hippocampus [153]. Saffron and crocin have been shown to prevent oxidative stress in the hippocampus and also prevent learning and memory deficits [138]. Crocetin has been reported to increase the antioxidant potential of the brain and to help counteract 6-OHDA-induced toxicity [154]. The aqueous extract of saffron prevented the increase of diazinon caused by increased inflammation, oxidative stress, and nerve damage markers [155].

7. Saffron and treatment of AD

7.1. Animal studies

Animal studies have demonstrated the beneficial action of saffron and crocin against AD (Table 1). As aforementioned, AD is pathologically characterized by the deposition of Aβ fibers in the brain. A recent *in vitro* study has shown that the water: methanol (50:50, v/v) extract of Crocus sativus stigmas showed good antioxidant properties, and inhibited Aβ fibrillogenesis in a concentration and time-dependent manner, suggesting the possible use of Crocus sativus stigma constituents in inhibition of aggregation and deposition of Aβ in the human brain [156]. Intraventricular injection of STZ in rodents is often utilized as an animal model for sporadic AD [157–159]. Crocin (30 mg/kg) can ameliorate spatial and associative learning and memory impairments in the STZ-induced model of sporadic AD in male rats [137]. Oral administration of crocin (100 mg/kg) for 21 days can prevent STZ- induced spatial memory deficit and oxidative stress in rats [160]. Inhibitors of

acetylcholine breakdown by AChE form the main therapeutic modality for AD. An *in vitro* enzymatic and molecular docking study has shown that saffron extract has moderate AChE inhibitory activity (up to 30 %), but IC50 values of crocetin, dimethylcrocetin, and safranal were 96.33, 107.1, and 21.09 μM, respectively. Safranal interacts only with the binding site of the AChE, but crocetin and dimethylcrocetin bind simultaneously to the catalytic and peripheral anionic sites. These results highlight the beneficial action of saffron against AD and may be of value for the development of novel therapeutic agents based on carotenoid-based dual binding inhibitors [161]. Acrolein, a by-product of lipid peroxidation, is involved in the pathogenesis of oxidative stress mediated neurodegenerative disorders such as AD as well as brain aging. A recent work has shown that oral administration of acrolein (3 mg/kg/day) decreased concentration of glutathione (GSH) and increased levels of MDA, β-amyloid and phosphor (p)-Tau in the rat brain cortex and simultaneously activated mitogen-activated protein kinases (MAPKs) signaling pathways. Co-treatment of crocin significantly ameliorated MDA, β-amyloid, and p-Tau levels by modulating MAPKs signaling pathways, highlighting the importance of crocin in the treatment of AD [162]. A recent study has shown that crocin can inhibit β-amyloid induced hippocampal apoptosis and spatial navigation impairment in the water maze in rats, showing crocin as a potential pharmaceutical agent for the management of AD [163]. An *in vitro* and *in vivo* recent study has shown that Crocus sativus extract increased the tightness of a cell-based blood-brain barrier and reduced Aβ load and related pathological changes in 5XFAD mice used as an AD model. Reduced Aβ load could be explained, at least in part, by Crocus sativus extract effect to enhance Aβ clearance pathways including blood-brain barrier clearance, enzymatic degradation, and ApoE clearance pathway [164]. Furthermore, Crocus sativus extract upregulated synaptic proteins and reduced neuro-inflammation associated with Aβ pathology in the brains of 5XFAD mice. Crocin (10 mg/kg/day) also was able to reduce Aβ load but to a lesser extent when compared to Crocus sativus extract. Collectively, findings of this study support the positive effect of Crocus sativus against AD by reducing Aβ pathological manifestations [164].

7.2. Human studies

Recent human studies have demonstrated the possible use of saffron in the treatment of AD (Table 1). A recent double-blind, placebo- controlled study on patients with mild to moderate AD has revealed that oral intake of 30 mg/day (15 mg twice per day) saffron for 16-weeks had a significantly better outcome on cognitive function than placebo with

Table 1
Preclinical (animal) and clinical (human) studies on the effects of saffron/crocin on memory/cognition in Alzheimer disease.

Effects	Subject	Drug Treatment	Route and duration of drug administration	Drug dose	Disease intensity	Duration and type of study	Reference
Crocin ameliorates spatial and associative learning and memory in in STZ-induced model of sporadic AD in male rats	Rat	Crocin	Systemic, 22 days	15 and 30 mg/kg	–	Experimental	[137]
Crocin prevents STZ induced spatial memory deficit and oxidative stress in rats	Rat	Crocin	Oral, 21 days	100 mg/kg 21 days	–	Experimental	[160]
Crocin inhibits beta amyloid induced hippocampal apoptosis and spatial navigation impairment in the water maze	Rat	Crocin Crocin	Intra-hippocampal, 20 days Systemic, 20 days	150,300 and 600 nmol/1μ 5 mg/kg	–	Experimental	[163]
Compared to placebo, saffron considerably ameliorated cognitive function in AD patients.	Human	Saffron extract	Oral, 16 weeks	30 mg/day	Mild - to -moderate AD	16 weeks, double-blind	[165]
Saffron was as efficient as donepezil and had few side influences in the treatment of AD patients	Human	Donepezil saffron extract	Oral, 22 weeks	10 mg/day 30 mg/day	Mild - to -moderate AD	22 weeks, double-blind	[166]
Saffron exhibits similar characteristics as memantine in preventing cognitive decline in AD patients.	Human	Memantine Saffron extract	Oral, 12 months	20 mg/day 30 mg/day	Moderate -to - severe AD	12 months, double blind	[167]
Patients using saffron experienced improved cognitive impairment, whereas the control group showed worsening conditions	Human	Saffron extract	Oral, 12 months	125 mg/day	Mild cognitive impairment	12 months, single-blind	[168]

no apparent adverse effects [165]. In another similar study, this dose of saffron was found to be effective similar to donepezil in the treatment of mild-to-moderate AD after 22 weeks [166]. Another randomized double-blind parallel-group study on 68 patients with moderate to severe AD showed that 1-year administration of saffron extract capsules (30 mg/kg) is comparable with memantine in reducing cognitive decline [167]. Another one-year efficacy and safety study of saffron in patients with mild cognitive impairment was recently conducted [168]. In this one-year study, 17 of 35 patients were included in the saffron group and 18 were in a parallel control group. Consequences demonstrated that patients on saffron group ameliorated cognitive impairment. These findings revealed that saffron could be efficacious in the improvement of mild cognitive impairment and AD. Although these data (Table 1) suggest that saffron could be a promising supplement or drug for prevention or treatment of AD, further studies with larger samples are required to evaluate long-term efficacy and safety of saffron as well as its optimal dosing for the management of AD.

8. Conclusion and future directions

AD progression is a multi-factorial process involving Aβ accumulation and Tau hyper-phosphorylation. Chronic stress and elevated GCs play an important role in the AD progression. Thus, restoring a normal functionality and activity of the HPA axis could be a striking approach and concrete way for new therapeutic strategies. Another effective way is targeting the outcomes of chronic stress resulting in AD progression such as Aβ and Tau proteins. The current drugs for AD treatment including AChE inhibitors and NMDA glutamate receptor blockers only provide modest benefits for delaying or preventing symptoms. During recent years, herbal medicine in conjunction with pharmacotherapy has gained much attention in the treatment of neurodegenerative diseases including AD, and Parkinson disease [169]. Recent studies on humans and experimental subjects

provided strong evidence that saffron and particularly its main ingredient, crocin have beneficial effects in delaying progression of AD in patients. They can inhibit the AChE activity, diminish corticosterone levels, modulate accumulation and aggregation of Aβ and Tau proteins, and reduce cognitive deficits including learning and memory [170,171]. Moreover, they could improve depression and anxiety which are symptoms frequently associated with AD patients. However, so far, a few animal and human clinical trial studies have investigated the beneficial effects of saffron and crocin on AD. Thus, further dose-response studies of saffron and crocin alone and in the combination with the current chemical drugs, and natural ingredients such as *Gingko Bulimia* [172] are essential to confirm their therapeutic and possible side effects in treatment of AD, and other stress-related disorders.

Declaration of Competing Interest

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References

[1] L. Jonsson, M.E. ¨ Jonhagen, L. Kilander, H. Soininen, M. Hallikainen,¨ G. Waldemar, H. Nygaard, N. Andreasen, B. Winblad, A. Wimo, Determinants of costs of care for patients with Alzheimer's disease, Int. J. Geriatric Psychiatr 21 (5) (2006) 449–459.

[2] L. Fratiglioni, L. Launer, K. Andersen, M. Breteler, J. Copeland, J.-F. Dartigues, A. Lobo, J. Martinez-Lage, H. Soininen, A. Hofman, Incidence of dementia and major subtypes in Europe: a collaborative study of population-based cohorts, Neurology 54 (11) (2000).

[3] A. Lobo, L. Launer, L. Fratiglioni, K. Andersen, A. Di Carlo, M. Breteler, J. Copeland, J. Dartigues, C. Jagger, J. Martinez-Lage, Prevalence of dementia and major subtypes in Europe: a collaborative study of population-based cohorts, Neurology 54 (5) (2000) S4.

[4] M. Citron, Strategies for disease modification in Alzheimer's disease, Nat. Rev. Neurosci. 5 (9) (2004) 677–685.

[5] D.J. Selkoe, Alzheimer's disease: genes, proteins, and therapy, Physiol. Rev. 81 (2) (2001) 741–766.

[6] F. Alipour, S. Oryan, M. Sharifzadeh, F. Karimzadeh, L. Kafami, H. Irannejad, M. Amini, G. Hassanzadeh, The Neuroprotective Effect of a Triazine Derivative in an Alzheimer's Rat Model, 2015.

[7] F. Alipour, F. Karimzadeh, G. Hasanzadeh, P59: Triazine improved hippocampal injuries in animal model of Alzheimer's disease, Neurosci. J. Shefaye Khatam 2 (4) (2014), 109-109.

[8] T.E. Golde, The aβ hypothesis: leading us to rationally-designed therapeutic strategies for the treatment or prevention of alzheimer disease, Brain Pathol. 15 (1) (2005) 84–87.

[9] F. Checler, Processing of the β-amyloid precursor protein and its regulation in Alzheimer's disease, J. Neurochem. 65 (4) (1995) 1431–1444.

[10] R.L. Neve, N.K. Robakis, Alzheimer's disease: a re-examination of the amyloid hypothesis, Trends Neurosci. 21 (1) (1998) 15–19.

[11] J. Di, L. Cohen, C. Corbo, G.R. Phillips, A. El Idrissi, A.D. Alonso, Abnormal tau induces cognitive impairment through two different mechanisms: synaptic dysfunction and neuronal loss, Sci. Rep. 6 (1) (2016) 1–12.

[12] C. Ballatore, V.M.-Y. Lee, J.Q. Trojanowski, Tau-mediated neurodegeneration in Alzheimer's disease and related disorders, Nat.

Rev. Neurosci. 8 (9) (2007) 663–672.
[13] R.A. Armstrong, Risk factors for Alzheimer's disease, Folia Neuropathol. 57 (2) (2019) 87–105.
[14] A. Henderson, The risk factors for Alzheimer's disease: a review and a hypothesis, Acta Psychiatr. Scand. 78 (3) (1988) 257–275.
[15] I. Sotiropoulos, N. Sousa, Tau as the converging protein between chronic stress and Alzheimer's disease synaptic pathology, Neurodegener. Dis. 16 (1-2) (2016) 22–25.
[16] S. Shadfar, C.J. Hwang, M.-S. Lim, D.-Y. Choi, J.T. Hong, Involvement of inflammation in Alzheimer's disease pathogenesis and therapeutic potential of anti-inflammatory agents, Arch. Pharm. Res. 38 (12) (2015) 2106–2119.
[17] X. Zhu, B. Su, X. Wang, M. Smith, G. Perry, Causes of oxidative stress in Alzheimer disease, Cell. Mol. Life Sci. 64 (17) (2007) 2202–2210.
[18] A. Nunomura, R.J. Castellani, X. Zhu, P.I. Moreira, G. Perry, M.A. Smith, Involvement of oxidative stress in Alzheimer disease, J. Neuropathol. Exp. Neurol. 65 (7) (2006) 631–641.
[19] C.D. Conrad, K.J. McLaughlin, J.S. Harman, C. Foltz, L. Wieczorek, E. Lightner, R. L. Wright, Chronic glucocorticoids increase hippocampal vulnerability to neurotoxicity under conditions that produce CA3 dendritic retraction but fail to impair spatial recognition memory, J. Neurosci. 27 (31) (2007) 8278–8285.
[20] A. Magarin, B. McEwen, Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: involvement of glucocorticoid secretion and excitatory amino acid receptors, Neuroscience 69 (1) (1995) 89–98.
[21] B.S. McEwen, Effects of adverse experiences for brain structure and function, Biol. Psychiatry 48 (8) (2000) 721–731.
[22] E. Gould, B.S. McEwen, P. Tanapat, L.A. Galea, E. Fuchs, Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation, J. Neurosci. 17 (7) (1997) 2492–2498.
[23] B.S. McEwen, Plasticity of the hippocampus: adaptation to chronic stress and allostatic load, Ann. N. Y. Acad. Sci. 933 (1) (2001) 265–277.
[24] C.D. Conrad, A critical review of chronic stress effects on spatial learning and memory, Prog. Neuropsychopharmacol. Biol. Psychiatry 34 (5) (2010) 742–755.
[25] I. Abidin, P. Yargicoglu, A. Agar, S. GÜMÜSLÜ, S. Aydin, S. Aydin, O. OZTÜRK, E. Sahin, The effect of chronic restraint stress on spatial learning and memory: relation to oxidant stress, Int. J. Neurosci. 114 (5) (2004) 683–699.
[26] I. Abraham, T. Harkany, K. Horvath, P. Luiten, Action of Glucocorticoids on Survival of Nerve Cells: Promoting Neurodegeneration or Neuroprotection? J. Neuroendocrinol. 13 (9) (2001) 749–760.
[27] L.J. McIntosh, R.M. Sapolsky, Glucocorticoids may enhance oxygen radical- mediated neurotoxicity, Neurotoxicology 17 (3-4) (1996) 873–882.
[28] R. Patel, L. McIntosh, J. McLaughlin, S. Brooke, V. Nimon, R. Sapolsky, Disruptive effects of glucocorticoids on glutathione peroxidase biochemistry in hippocampal cultures, J. Neurochem. 82 (1) (2002) 118–125.
[29] G. Storz, J.A. Imlayt, Oxidative stress, Curr. Opin. Microbiol. 2 (2) (1999) 188–194.
[30] M.-F. Marin, C. Lord, J. Andrews, R.-P. Juster, S. Sindi, G. Arsenault-Lapierre, A. J. Fiocco, S.J. Lupien, Chronic stress, cognitive functioning and mental health, Neurobiol. Learn. Mem. 96 (4) (2011) 583–595.
[31] J.P. Herman, J.M. McKlveen, S. Ghosal, B. Kopp, A. Wulsin, R. Makinson, J. Scheimann, B. Myers, Regulation of the hypothalamic-pituitary-adrenocortical stress response, Compr. Physiol. 6 (2) (2011) 603–621.
[32] I. Chaudieu, I. Beluche, J. Norton, J.-P. Boulenger, K. Ritchie, M.L. Ancelin, Abnormal reactions to environmental stress in elderly persons with anxiety disorders: evidence from a population study of diurnal cortisol changes, J. Affect. Disord. 106 (3) (2008) 307–313.
[33] C. De Kloet, E. Vermetten, E. Geuze, A. Kavelaars, C. Heijnen, H. Westenberg,

Assessment of HPA-axis function in posttraumatic stress disorder: pharmacological and non-pharmacological challenge tests, a review, J. Psychiatr. Res. 40 (6) (2006) 550–567.

[34] G. Garcia-Banda, K. Chellew, J. Fornes, G. Perez, M. Servera, P. Evans, Neuroticism and cortisol: pinning down an expected effect, Int. J. Psychophysiol. 91 (2) (2014) 132–138.

[35] G.E. Ennis, Y. An, S.M. Resnick, L. Ferrucci, R.J. O'Brien, S.D. Moffat, Long-term cortisol measures predict Alzheimer disease risk, Neurology 88 (4) (2017) 371–378.

[36] R. Libro, P. Bramanti, E. Mazzon, Endogenous glucocorticoids: role in the etiopathogenesis of Alzheimer's disease, Neuroendocrinol. Lett 38 (2017) 1–12.

[37] J.G. Csernansky, H. Dong, A.M. Fagan, L. Wang, C. Xiong, D.M. Holtzman, J. C. Morris, Plasma cortisol and progression of dementia in subjects with Alzheimer-type dementia, Am. J. Psychiatry 163 (12) (2006) 2164–2169.

[38] J. Popp, S. Wolfsgruber, I. Heuser, O. Peters, M. Hüll, J. Schroder, H.-J. ¨ Moller,¨ P. Lewczuk, A. Schneider, H. Jahn, Cerebrospinal fluid cortisol and clinical disease progression in MCI and dementia of Alzheimer's type, Neurobiol. Aging 36 (2) (2015) 601–607.

[39] F.J. Gil-Bea, B. Aisa, A. Solomon, M. Solas, M. del Carmen Mugueta, B. Winblad, M. Kivipelto, A. Cedazo-Minguez, M.J. Ramírez, HPA axis dysregulation associated to apolipoprotein E4 genotype in Alzheimer's disease, J. Alzheimer Dis. 22 (3) (2010) 829–838.

[40] G.M. Peavy, D.P. Salmon, M.W. Jacobson, A. Hervey, A.C. Gamst, T. Wolfson, T. L. Patterson, S. Goldman, P.J. Mills, S. Khandrika, Effects of chronic stress on memory decline in cognitively normal and mildly impaired older adults, Am. J. Psychiatry 166 (12) (2009) 1384–1391.

[41] E. Schrijvers, N. Direk, P.J. Koudstaal, C. Kirschbaum, A. Hofman, H. Tiemeier, M. Breteler, Associations of serum cortisol with cognitive function and dementia: the Rotterdam Study, J. Alzheimer's Dis. 25 (4) (2011) 671–677.

[42] K.N. Green, L.M. Billings, B. Roozendaal, J.L. McGaugh, F.M. LaFerla, Glucocorticoids increase amyloid-β and tau pathology in a mouse model of Alzheimer's disease, J. Neurosci. 26 (35) (2006) 9047–9056.

[43] M.E. Harris-White, T. Chu, S.A. Miller, M. Simmons, B. Teter, D. Nash, G.M. Cole, S.A. Frautschy, Estrogen (E2) and glucocorticoid (Gc) effects on microglia and Aβ clearance in vitro and in vivo, Neurochem. Int. 39 (5-6) (2001) 435–448.

[44] I. Sotiropoulos, C. Catania, L.G. Pinto, R. Silva, G.E. Pollerberg, A. Takashima, N. Sousa, O.F. Almeida, Stress acts cumulatively to precipitate Alzheimer's disease-like tau pathology and cognitive deficits, J. Neurosci. 31 (21) (2011) 7840–7847.

[45] H. Zempel, E. Mandelkow, Lost after translation: missorting of Tau protein and consequences for Alzheimer disease, Trends Neurosci. 37 (12) (2014) 721–732. [46] R.J. Bateman, C. Xiong, T.L. Benzinger, A.M. Fagan, A. Goate, N.C. Fox, D. S. Marcus, N.J. Cairns, X. Xie, T.M. Blazey, Clinical and biomarker changes in dominantly inherited Alzheimer's disease, N. Engl. J. Med. 367 (2012) 795–804. [47] W.J. Jansen, R. Ossenkoppele, D.L. Knol, B.M. Tijms, P. Scheltens, F.R. Verhey, P. J. Visser, P. Aalten, D. Aarsland, D. Alcolea, Prevalence of cerebral amyloid pathology in persons without dementia: a meta-analysis, JAMA 313 (19) (2015) 1924–1938.

[48] R.A. Sperling, P.S. Aisen, L.A. Beckett, D.A. Bennett, S. Craft, A.M. Fagan, T. Iwatsubo, C.R. Jack Jr, J. Kaye, T.J. Montine, Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease, Alzheimer's Dementia 7 (3) (2011) 280–292.

[49] K.A. Alkadhi, Chronic stress and Alzheimer's disease-like pathogenesis in a rat model: prevention by nicotine, Curr. Neuropharmacol. 9 (4) (2011) 587–597.

[50] M. Srivareerat, T.T. Tran, K.H. Alzoubi, K.A. Alkadhi, Chronic psychosocial stress exacerbates impairment of cognition and long-term potentiation in β-amyloid rat model of Alzheimer's disease, Biol. Psychiatry 65 (11) (2009) 918–926.

[51] N.J. Justice, The relationship between stress and

Alzheimer's disease, Neurobiol. Stress 8 (2018) 127–133.
[52] D. Costantini, V. Marasco, A.P. Møller, A meta-analysis of glucocorticoids as modulators of oxidative stress in vertebrates, J. Comp. Physiol. B 181 (4) (2011) 447–456.
[53] P.K. Kamat, A. Kalani, S. Rai, S. Swarnkar, S. Tota, C. Nath, N. Tyagi, Mechanism of oxidative stress and synapse dysfunction in the pathogenesis of Alzheimer's disease: understanding the therapeutics strategies, Mol. Neurobiol. 53 (1) (2016) 648–661.
[54] S. Schiavone, V. Jaquet, L. Trabace, K.-H. Krause, Severe life stress and oxidative stress in the brain: from animal models to human pathology, Antioxid. Redox Signal. 18 (12) (2013) 1475–1490.
[55] W. Gsell, I. Strein, P. Riederer, The neurochemistry of Alzheimer type, vascular type and mixed type dementias compared. New Trends in the Diagnosis and Therapy of Non-Alzheimer's Dementia, Springer, 1996, pp. 73–101.
[56] W. Gsell, I. Strein, U. Krause, P. Riederer, Neurochemical abnormalities in Alzheimer's disease and Parkinson's disease—a comparative review. Dementia in Parkinsonism, Springer, 1997, pp. 145–159.
[57] W. Retz, W. Gsell, G. Münch, M. Rosler, P. Riederer, Free radicals in Alzheime¨ r's disease. Alzheimer's Disease—From Basic Research to Clinical Applications, Springer, 1998, pp. 221–236.
[58] M. Rosler, W. Retz, J. Thome, P. Riederer, Free radicals in Alzheime¨ r's dementia: currently available therapeutic strategies. Alzheimer's Disease—From Basic Research to Clinical Applications, Springer, 1998, pp. 211–219.
[59] N. Durany, G. Münch, T. Michel, P. Riederer, Investigations on oxidative stress and therapeutical implications in dementia, Eur. Arch. Psychiatry Clin. Neurosci. 249 (3) (1999) S68–S73.
[60] Y. Christen, Oxidative stress and Alzheimer disease, Am. J. Clin. Nutr. 71 (2) (2000) 621S–629S.
[61] K.J. Barnham, C.L. Masters, A.I. Bush, Neurodegenerative diseases and oxidative stress, Nat. Rev. Drug Discov. 3 (3) (2004) 205–214.
[62] L.M. Sayre, P.I. Moreira, M.A. Smith, G. Perry, Metal ions and oxidative protein modification in neurological disease, Annali dell'Istituto superiore di sanita 41 (2) (2005) 143–164.
[63] L.M. Sayre, G. Perry, M.A. Smith, Oxidative stress and neurotoxicity, Chem. Res. Toxicol. 21 (1) (2008) 172–188.
[64] M.J. Forster, A. Dubey, K.M. Dawson, W.A. Stutts, H. Lal, R.S. Sohal, Age-related losses of cognitive function and motor skills in mice are associated with oxidative protein damage in the brain, Proc. Natl. Acad. Sci. 93 (10) (1996) 4765–4769.
[65] S. Schippling, A. Kontush, S. Arlt, C. Buhmann, H.-J. Stürenburg, U. Mann, T. Müller-Thomsen, U. Beisiegel, Increased lipoprotein oxidation in Alzheimer's disease, Free Radic. Biol. Med. 28 (3) (2000) 351–360.
[66] M.M.A. El Mohsen, M.M. Iravani, J.P. Spencer, S. Rose, A.T. Fahim, T.M. Motawi, N.A. Ismail, P. Jenner, Age-associated changes in protein oxidation and proteasome activities in rat brain: modulation by antioxidants, Biochem. Biophys. Res. Commun. 336 (2) (2005) 386–391.
[67] M.A. Smith, R.K. Kutty, P.L. Richey, S.-D. Yan, D. Stern, G.J. Chader, B. Wiggert, R.B. Petersen, G. Perry, Heme oxygenase-1 is associated with the neurofibrillary pathology of Alzheimer's disease, Am. J. Pathol. 145 (1) (1994) 42.
[68] A. Gupta, M. Hasan, R. Chander, N.K. Kapoor, Age-related elevation of lipid peroxidation products: diminution of superoxide dismutase activity in the central nervous system of rats, Gerontology 37 (6) (1991) 305–309.
[69] M. Cini, A. Moretti, Studies on lipid peroxidation and protein oxidation in the aging brain, Neurobiol. Aging 16 (1) (1995) 53–57.
[70] C. Ramassamy, P. Krzywkowski, D. Averill, S. Lussier-Cacan, L. Theroux, Y. Christen, J. Davignon, J. Poirier, Impact of apoE deficiency on oxidative insults and antioxidant levels in the brain, Mol. Brain Res. 86 (1-2) (2001) 76–83.
[71] M.J. Picklo Sr, T.J. Montine, V. Amarnath, M.D. Neely, Carbonyl toxicology and Alzheimer's disease, Toxicol. Appl. Pharmacol. 184 (3) (2002) 187–197.
[72] C. Bassett, T.J. Montine, Lipoproteins and lipid

peroxidation in Alzheimer's disease, J. Nutrition Health Aging 7 (1) (2003) 24–29.
[73] P. Mecocci, M.F. Beal, R. Cecchetti, M.C. Polidori, A. Cherubini, F. Chionne, L. Avellini, G. Romano, U. Senin, Mitochondrial membrane fluidity and oxidative damage to mitochondrial DNA in aged and AD human brain, Mol. Chem. Neuropathol. 31 (1) (1997) 53–64.
[74] M.L. Hamilton, H. Van Remmen, J.A. Drake, H. Yang, Z.M. Guo, K. Kewitt, C.
A. Walter, A. Richardson, Does oxidative damage to DNA increase with age? Proc. Natl. Acad. Sci. 98 (18) (2001) 10469–10474.
[75] M.A. Lovell, W.R. Markesbery, Oxidative DNA damage in mild cognitive impairment and late-stage Alzheimer's disease, Nucleic Acids Res. 35 (22) (2007) 7497–7504.
[76] W.J. Perrig, P. Perrig, H.B. Stahelin, The relation between antioxidants and¨ memory performance in the old and very old, J. Am. Geriatr. Soc. 45 (6) (1997) 718–724.
[77] C. Berr, Cognitive impairment and oxidative stress in the elderly: results of epidemiological studies, Biofactors 13 (1-4) (2000) 205–209.
[78] P.J. Thornalley, Glutathione-dependent detoxification of α-oxoaldehydes by the glyoxalase system: involvement in disease mechanisms and antiproliferative activity of glyoxalase I inhibitors, Chem. Biol. Interact. 111 (1998) 137–151.
[79] T.S. Chen, J.P. Richie Jr, C.A. Lang, The effect of aging on glutathione and cysteine levels in different regions of the mouse brain, Proc. Soc. Exp. Biol. Med.
190 (4) (1989) 399–402.
[80] T. Iantomasi, F. Favilli, P. Marraccini, M. Stio, C. Treves, A. Quatrone, S. Capaccioli, M.T. Vincenzini, Age and GSH metabolism in rat cerebral cortex, as related to oxidative and energy parameters, Mech. Ageing Dev. 70 (1-2) (1993) 65–82.
[81] T. Sasaki, M. Senda, S.-n. Kim, S. Kojima, A. Kubodera, Age-related changes of glutathione content, glucose transport and metabolism, and mitochondrial electron transfer function in mouse brain, Nucl. Med. Biol. 28 (1) (2001) 25–31.
[82] R.M. Liu, Down-regulation of γ-glutamylcysteine synthetase regulatory subunit gene expression in rat brain tissue during aging, J. Neurosci. Res. 68 (3) (2002) 344–351.
[83] S.K. Sandhu, G. Kaur, Alterations in oxidative stress scavenger system in aging rat brain and lymphocytes, Biogerontology 3 (3) (2002) 161–173.
[84] H. Wang, H. Liu, R.-M. Liu, Gender difference in glutathione metabolism during aging in mice, Exp. Gerontol. 38 (5) (2003) 507–517.
[85] V. Calabrese, G. Scapagnini, A. Ravagna, C. Colombrita, F. Spadaro, D. Butterfield, A.G. Stella, Increased expression of heat shock proteins in rat brain during aging: relationship with mitochondrial function and glutathione redox state, Mech. Ageing Dev. 125 (4) (2004) 325–335.
[86] A.N. Donahue, M. Aschner, L.H. Lash, T. Syversen, W.E. Sonntag, Growth hormone administration to aged animals reduces disulfide glutathione levels in hippocampus, Mech. Ageing Dev. 127 (1) (2006) 57–63.
[87] Y. Zhu, P.M. Carvey, Z. Ling, Age-related changes in glutathione and glutathione- related enzymes in rat brain, Brain Res. 1090 (1) (2006) 35–44.
[88] W. Gsell, R. Conrad, M. Hickethier, E. Sofic, L. Frolich, I. Wichart, K. Jellinger,¨ G. Moll, G. Ransmayr, H. Beckmann, Decreased catalase activity but unchanged superoxide dismutase activity in brains of patients with dementia of Alzheimer type, J. Neurochem. 64 (3) (1995) 1216–1223.
[89] P. Mecocci, U. MacGarvey, M.F. Beal, Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease, Ann. Neurol. 36 (5) (1994) 747–751. [90] K. Hensley, N. Hall, R. Subramaniam, P. Cole, M. Harris, M. Aksenov, M. Aksenova, S.P. Gabbita, J.F. Wu, J.M. Carney, Brain regional correspondence between Alzheimer's disease histopathology and biomarkers of protein oxidation, J. Neurochem. 65 (5) (1995) 2146–2156.
[91] A.M. Palmer, M.A. Burns, Selective increase in lipid peroxidation in the inferior temporal cortex in Alzheimer's disease, Brain Res. 645 (1-2) (1994) 338–342.

[92] M.A. Lovell, W.D. Ehmann, S.M. Butler, W.R. Markesbery, Elevated thiobarbituric acid-reactive substances and antioxidant enzyme activity in the brain in Alzheimer's disease, Neurology 45 (8) (1995) 1594–1601.
[93] D.L. Marcus, C. Thomas, C. Rodriguez, K. Simberkoff, J.S. Tsai, J.A. Strafaci, M. L. Freedman, Increased peroxidation and reduced antioxidant enzyme activity in Alzheimer's disease, Exp. Neurol. 150 (1) (1998) 40–44.
[94] O. Lazarov, R.A. Marr, Neurogenesis and Alzheimer's disease: at the crossroads, Exp. Neurol. 223 (2) (2010) 267–281.
[95] I. Ziabreva, E. Perry, R. Perry, S.L. Minger, A. Ekonomou, S. Przyborski, C. Ballard, Altered neurogenesis in Alzheimer's disease, J. Psychosom. Res. 61 (3) (2006) 311–316.
[96] G. Münch, R. Schinzel, C. Loske, A. Wong, N. Durany, J. Li, H. Vlassara, M. Smith, G. Perry, P. Riederer, Alzheimer's disease–synergistic effects of glucose deficit, oxidative stress and advanced glycation endproducts, J. Neural Transm. 105 (4-5) (1998) 439–461.
[97] G.L. Wenk, Neuropathologic changes in Alzheimer's disease: potential targets for treatment, J. Clin. Psychiatry 67 (2006) 3.
[98] B. McGleenon, K. Dynan, A. Passmore, Acetylcholinesterase inhibitors in Alzheimer's disease, Br. J. Clin. Pharmacol. 48 (4) (1999) 471.
[99] W. Danysz, C.G. Parsons, The NMDA receptor antagonist memantine as a symptomatological and neuroprotective treatment for Alzheimer's disease: preclinical evidence, Int. J. Geriatr. Psychiatry 18 (S1) (2003) S23–S32.
[100] M. Mehta, A. Adem, M. Sabbagh, New acetylcholinesterase inhibitors for Alzheimer's disease, Int. J. Alzheimer's Dis. 2012 (2012).
[101] P.N. Tariot, M.R. Farlow, G.T. Grossberg, S.M. Graham, S. McDonald, I. Gergel, M. S. Group, Memantine treatment in patients with moderate to severe Alzheimer disease already receiving donepezil: a randomized controlled trial, JAMA 291 (3) (2004) 317–324.
[102] D. Mantle, A.T. Pickering, E.K. Perry, Medicinal plant extracts for the treatment of dementia, CNS Drugs 13 (3) (2000) 201–213.
[103] A.A. Izzo, F. Capasso, Herbal medicines to treat Alzheimer's disease, Trends Pharmacol. Sci. 2 (28) (2007) 47–48.
[104] M. Thakur, N. Sharma, Saffron: a golden condiment and a repository of nutraceutical potential, Food Sci. Res. J. 5 (2014) 59–67.
[105] B.A. Wani, A.K.R. Hamza, F. Mohiddin, Saffron: A repository of medicinal properties, J. Med. Plant Res. 5 (11) (2011) 2131–2135.
[106] K. Abe, H. Saito, Effects of saffron extract and its constituent crocin on learning behaviour and long-term potentiation, Phytother. Res. 14 (3) (2000) 149–152.
[107] N.S. Hagh, N. Keyfi, Saffron and various fraud matter in its production and trade, Proceedings of International Symposium on Saffron Biology and Technology (2006).
[108] S.S. MH, Medicinal Plants and Phytotherapy, The World of Nutrition Publications, Iran, 2006, pp. 207–210.
[109] S. Verma, A. Bordia, Antioxidant property of saffron in man, Indian J. Med. Sci. 52 (5) (1998) 205–207.
[110] S. Akhondzadeh, N. Tahmacebi-Pour, A.A. Noorbala, H. Amini, H. Fallah-Pour, A. H. Jamshidi, M. Khani, Crocus sativus L. In the treatment of mild to moderate depression: a double-blind, randomized and placebo-controlled trial, Phytother. Res. 19 (2) (2005) 148–151.
[111] S. Akhoundzadeh, H. Falahpour, K. Afkham, A. Jamshidi, C.F. KHALIGHI, L. Miller, A Comparative Trial of Crocus sativus L. (saffron) and Imipramine in Mild to Moderate Depression, 2005.
[112] A. Noorbala, S. Akhondzadeh, N. Tahmacebi-Pour, A. Jamshidi, Hydro-alcoholic extract of Crocus sativus L. versus fluoxetine in the treatment of mild to moderate depression: a double-blind, randomized pilot trial, J. Ethnopharmacol. 97 (2) (2005) 281–284.
[113] S. Kianbakht, A. Ghazavi, Evaluation of Immunological and Hematological Effects of Saffron in Men, 2005.
[114] M. Agha-Hosseini, L. Kashani, A. Aleyaseen, A. Ghoreishi, H. Rahmanpour, A. Zarrinara, S. Akhondzadeh, Crocus

sativus L.(saffron) in the treatment of premenstrual syndrome: a double-blind, randomised and placebo-controlled trial, BJOG Int. J. Obstet. Gynaecol. 115 (4) (2008) 515–519.
[115] D.A. L.-K.M.a.K, Crocus sativus biological active constituents, Stud. Nat. Prod. Chem. 26 (7) (2002) 293–312.
[116] N. Pitsikas, S. Zisopoulou, P.A. Tarantilis, C.D. Kanakis, M.G. Polissiou, N. Sakellaridis, Effects of the active constituents of Crocus sativus L., crocins on recognition and spatial rats' memory, Behav. Brain Res. 183 (2) (2007) 141–146.
[117] J. Rios, M. Recio, R. Giner, S. Manez, An update review of saffron and its active constituents, Phytother. Res. 10 (3) (1996) 189–193.
[118] F. Abdullaev, J. Espinosa-Aguirre, Biomedical properties of saffron and its potential use in cancer therapy and chemoprevention trials, Cancer Detect. Prev. 28 (6) (2004) 426–432.
[119] H. Hosseinzadeh, A. Abootorabi, H.R. Sadeghnia, Protective effect of Crocus sativus stigma extract and crocin (trans-crocin 4) on methyl methanesulfonate–induced DNA damage in mice organs, DNA Cell Biol. 27 (12) (2008) 657–664.
[120] K. Premkumar, S.K. Abraham, S. Santhiya, A. Ramesh, Protective effects of saffron (Crocus sativus Linn.) on genotoxins-induced oxidative stress in Swiss albino mice, Phytother. Res. 17 (6) (2003) 614–617.
[121] H. Hosseinzadeh, V. Khosravan, Anticonvulsant Effects of Aqueous Ana Ethanolic Extracts of Crocus Sativus L Stigmas in Mice, 2002.
[122] H. Hosseinzadeh, G. Karimi, M. Niapoor, Antidepressant effects of Crocus sativus stigma extracts and its constituents, crocin and safranal, in mice, J. Med. Plants 3 (11) (2004) 48–58.
[123] H. Hosseinzadeh, H.M. Younesi, Antinociceptive and anti-inflammatory effects of Crocus sativus L. stigma and petal extracts in mice, BMC Pharmacol. 2 (1) (2002) 7.
[124] F.I. Abdullaev, Biological effects of saffron, BioFactors (Oxford, England) 4 (2) (1993) 83–86.
[125] J. Escribano, G.-L. Alonso, M. Coca-Prados, J.-A. Fernandez, Crocin, safranal and´ picrocrocin from saffron (Crocus sativus L.) inhibit the growth of human cancer cells in vitro, Cancer Lett. 100 (1-2) (1996) 23–30.
[126] Y. Zhang, Y. Shoyama, M. Sugiura, H. Saito, Effects of Crocus sativus L. on the ethanol-induced impairment of passive avoidance performances in mice, Biol. Pharm. Bull. 17 (2) (1994) 217–221.
[127] K. Abe, M. Sugiura, S. Yamaguchi, Y. Shoyama, H. Saito, Saffron extract prevents acetaldehyde-induced inhibition of long-term potentiation in the rat dentate gyrus in vivo, Brain Res. 851 (1-2) (1999) 287–289.
[128] F.J. Abdullaev, H. Caballero-Ortega, L. Riveron-Negrete, R. Pereda-Miranda, R. Rivera-Luna, J.H. Manuel, I. Perez-Lopez, J. Espinosa-Aguirre, In vitro evaluation of the chemopreventive potential of saffron, Revista de investigacion clinica; organo del Hospital de Enfermedades de la Nutricion 54 (5) (2002) 430–436.
[129] S.C. Nair, S. Kurumboor, J. Hasegawa, Saffron chemoprevention in biology and medicine: a review, Cancer Biother. Radiopharm. 10 (4) (1995) 257–264.
[130] K. Premkumar, S.K. Abraham, S. Santhiya, P. Gopinath, A. Ramesh, Inhibition of genotoxicity by saffron (Crocus sativus L.) in mice, Drug Chem. Toxicol. 24 (4) (2001) 421–428.
[131] A. Hensel, M. Niehues, M. Lechtenberg, B. Quandt, D. Schepmann, B. Wünsch, Analytical and functional aspects on Saffron from Crocus sativus L.: development of quality control methods, species assortment and affinity to sigma-1 and NMDA receptors, Planta Med. 72 (11) (2006) P_074.
[132] K. Abe, M. Sugiura, Y. Shoyama, H. Saito, Crocin antagonizes ethanol inhibition of NMDA receptor-mediated responses in rat hippocampal neurons, Brain Res. 787 (1) (1998) 132–138.
[133] G. Collingridge, T. Bliss, Memories of NMDA receptors and LTP, Trends Neurosci. 18 (2) (1995) 54–56.
[134] M. Sugiura, H. Saito, K. Abe, Y. Shoyama, Ethanol extract of Crocus sativus L. Antagonizes the inhibitory action of

ethanol on hippocampal long-term potentiation in vivo, Phytother. Res. 9 (2) (1995) 100–104.
[135] H. Hosseinzadeh, T. Ziaei, Effects of Crocus sativus stigma extract and its constituents, crocin and safranal, on intact memory and scopolamine-induced learning deficits in rats performing the Morris water maze task, J. Med. Plants 3 (19) (2006) 40–50.
[136] N. Pitsikas, N. Sakellaridis, Crocus sativus L. extracts antagonize memory impairments in different behavioural tasks in the rat, Behav. Brain Res. 173 (1) (2005) 112–115.
[137] M. Khalili, F. Hamzeh, Effects of active constituents of Crocus sativus L., crocin on streptozocin-induced model of sporadic Alzheimer's disease in male rats, Iran. Biomed. J. 14 (1-2) (2010) 59.
[138] B. Ghadrdoost, A.A. Vafaei, A. Rashidy-Pour, R. Hajisoltani, A.R. Bandegi, F. Motamedi, S. Haghighi, H.R. Sameni, S. Pahlvan, Protective effects of saffron extract and its active constituent crocin against oxidative stress and spatial learning and memory deficits induced by chronic stress in rats, Eur. J. Pharmacol. 667 (1-3) (2011) 222–229.
[139] M. Ghalandari-Shamami, S. Nourizade, B. Yousefi, A.A. Vafaei, R. Pakdel, A. Rashidy-Pour, Beneficial effects of physical activity and crocin against adolescent stress induced anxiety or depressive-like symptoms and dendritic morphology remodeling in prefrontal cortex in adult male rats, Neurochem. Res. 44 (4) (2019) 917–929.
[140] Y. Chen, K. Brunson, G. Adelmann, R. Bender, M. Frotscher, T. Baram, Hippocampal corticotropin releasing hormone: pre- and postsynaptic location and release by stress, Neuroscience 126 (3) (2004) 533–540.
[141] S. Asalgoo, M. Tat, H. Sahraei, G. Pirzad Jahromi, The psychoactive agent crocin can regulate hypothalamic-pituitary-adrenal axis activity, Front. Neurosci. 11 (2017) 668.
[142] A.R. Bandegi, A. Rashidy-Pour, A.A. Vafaei, B. Ghadrdoost, Protective effects of Crocus sativus L. extract and crocin against chronic-stress induced oxidative damage of brain, liver and kidneys in rats, Adv. Pharm. Bull. 4 (Suppl. 2) (2014) 493.
[143] Z. Hooshmandi, A.H. Rohani, A. Eidi, Z. Fatahi, L. Golmanesh, H. Sahraei, Reduction of metabolic and behavioral signs of acute stress in male Wistar rats by saffron water extract and its constituent safranal, Pharm. Biol. 49 (9) (2011) 947–954.
[144] Ba.S. Halataei, M. Khosravi, S. Arbabian, H. Sahraei, L. Golmanesh, H. Zardooz, C. Jalili, H. Ghoshooni, Saffron (Crocus sativus) aqueous extract and its constituent crocin reduces stress-induced anorexia in mice, Phytother. Res. 25 (12) (2011) 1833–1838.
[145] S. Saleem, M. Ahmad, A.S. Ahmad, S. Yousuf, M.A. Ansari, M.B. Khan, T. Ishrat, F. Islam, Effect of Saffron (Crocus sativus) on neurobehavioral and neurochemical changes in cerebral ischemia in rats, J. Med. Food 9 (2) (2006) 246–253.
[146] S.H. Mousavi, N. Tayarani, H. Parsaee, Protective effect of saffron extract and crocin on reactive oxygen species-mediated high glucose-induced toxicity in PC12 cells, Cell. Mol. Neurobiol. 30 (2) (2010) 185–191.
[147] A. Shati, F. Elsaid, E. Hafez, Biochemical and molecular aspects of aluminium chloride-induced neurotoxicity in mice and the protective role of Crocus sativus L. extraction and honey syrup, Neuroscience 175 (2011) 66–74.
[148] H. Hosseinzadeh, H.R. Sadeghnia, Safranal, a constituent of Crocus sativus (saffron), attenuated cerebral ischemia induced oxidative damage in rat hippocampus, J. Pharm. Pharm. Sci. 8 (3) (2005) 394–399.
[149] H.R. Sadeghnia, M. Kamkar, E. Assadpour, M.T. Boroushaki, A. Ghorbani, Protective effect of safranal, a constituent of Crocus sativus, on quinolinic acid- induced oxidative damage in rat hippocampus, Iran. J. Basic Med. Sci. 16 (1) (2013) 73.
[150] H. Hosseinzadeh, H.R. Sadeghnia, A. Rahimi, Effect of safranal on extracellular hippocampal levels of glutamate and aspartate during kainic acid treatment in anesthetized rats, Planta Med. 74 (12) (2008) 1441–1445.
[151] A. Vakili, M.R. Einali, A.R. Bandegi, Protective effect of crocin against cerebral

ischemia in a dose-dependent manner in a rat model of ischemic stroke, J. Stroke Cerebrovasc. Dis. 23 (1) (2014) 106–113.
[152] Z.I. Linardaki, M.G. Orkoula, A.G. Kokkosis, F.N. Lamari, M. Margarity, Investigation of the neuroprotective action of saffron (Crocus sativus L.) in aluminum-exposed adult mice through behavioral and neurobiochemical assessment, Food Chem. Toxicol. 52 (2013) 163–170.
[153] C. Behl, F. Lezoualc'h, T. Trapp, M. Widmann, T. Skutella, F. Holsboer, Glucocorticoids enhance oxidative stress-induced cell death in hippocampal neurons in vitro, Endocrinology 138 (1) (1997) 101–106.
[154] A.S. Ahmad, M.A. Ansari, M. Ahmad, S. Saleem, S. Yousuf, M.N. Hoda, F. Islam, Neuroprotection by crocetin in a hemi-parkinsonian rat model, Pharmacol. Biochem. Behav. 81 (4) (2005) 805–813.
[155] S.A. Moallem, A.T. Hariri, M. Mahmoudi, H. Hosseinzadeh, Effect of aqueous extract of Crocus sativus L.(saffron) stigma against subacute effect of diazinon on specific biomarkers in rats, Toxicol. Ind. Health 30 (2) (2014) 141–146.
[156] M.A. Papandreou, C.D. Kanakis, M.G. Polissiou, S. Efthimiopoulos, P. Cordopatis, M. Margarity, F.N. Lamari, Inhibitory activity on amyloid-β aggregation and antioxidant properties of Crocus sativus stigmas extract and its crocin constituents, J. Agric. Food Chem. 54 (23) (2006) 8762–8768.
[157] H. Lannert, S. Hoyer, Intracerebroventricular administration of streptozotocin causes long-term diminutions in learning and memory abilities and in cerebral energy metabolism in adult rats, Behav. Neurosci. 112 (5) (1998) 1199.
[158] M. Labak, T. Foniok, D. Kirk, D. Rushforth, B. Tomanek, A. Jasinski, P. Grieb, ´ Metabolic Changes in Rat Brain Following Intracerebroventricular Injections of Streptozotocin: A Model of Sporadic Alzheimer's Disease, Brain Edema XIV, Springer, 2010, pp. 177–181.
[159] M. Veerendra Kumar, Y. Gupta, Effect of Centella asiatica on cognition and oxidative stress in an intracerebroventricular streptozotocin model of Alzheimer's disease in rats, Clin. Exp. Pharmacol. Physiol. 30 (5-6) (2003) 336–342.
[160] B. Naghizadeh, M. Mansouri, B. Ghorbanzadeh, Y. Farbood, A. Sarkaki, Protective effects of oral crocin against intracerebroventricular streptozotocin-induced spatial memory deficit and oxidative stress in rats, Phytomedicine 20 (6) (2013) 537–542.
[161] G.D. Geromichalos, F.N. Lamari, M.A. Papandreou, D.T. Trafalis, M. Margarity, A. Papageorgiou, Z. Sinakos, Saffron as a source of novel acetylcholinesterase inhibitors: molecular docking and in vitro enzymatic studies, J. Agric. Food Chem. 60 (24) (2012) 6131–6138.
[162] M. Rashedinia, P. Lari, K. Abnous, H. Hosseinzadeh, Protective effect of crocin on acrolein-induced tau phosphorylation in the rat brain, Acta Neurobiol. Exp. 75 (2) (2015) 208–219.
[163] F. Asadi, A.H. Jamshidi, F. Khodagholi, A. Yans, L. Azimi, M. Faizi, L. Vali, M. Abdollahi, M.H. Ghahremani, M. Sharifzadeh, Reversal effects of crocin on amyloid β-induced memory deficit: modification of autophagy or apoptosis markers, Pharmacol. Biochem. Behav. 139 (2015) 47–58.
[164] Y.S. Batarseh, S.S. Bharate, V. Kumar, A. Kumar, R.A. Vishwakarma, S.B. Bharate, A. Kaddoumi, Crocus sativus extract tightens the blood-brain barrier, reduces amyloid β load and related toxicity in 5XFAD mice, ACS Chem. Neurosci. 8 (8) (2017) 1756–1766.
[165] S. Akhondzadeh, M.S. Sabet, M. Harirchian, M. Togha, H. Cheraghmakani, S. Razeghi, S.S. Hejazi, M. Yousefi, R. Alimardani, A. Jamshidi, Saffron in the treatment of patients with mild to moderate Alzheimer's disease: a 16-week, randomized and placebo-controlled trial, J. Clin. Pharm. Ther. 35 (5) (2010) 581–588.
[166] S. Akhondzadeh, M.S. Sabet, M.H. Harirchian, M. Togha, H. Cheraghmakani, S. Razeghi, S.S. Hejazi, M.H. Yousefi, R. Alimardani, A. Jamshidi, A 22-week, multicenter, randomized, double-blind controlled trial of Crocus sativus in the treatment of

mild-to-moderate Alzheimer's disease, Psychopharmacology 207 (4) (2010) 637–643.
[167] M. Farokhnia, M. Shafiee Sabet, N. Iranpour, A. Gougol, H. Yekehtaz, R. Alimardani, F. Farsad, M. Kamalipour, S. Akhondzadeh, Comparing the efficacy and safety of Crocus sativus L. with memantine in patients with moderate to severe Alzheimer's disease: a double-blind randomized clinical trial, Hum. Psychopharmacol. Clin. Exp. 29 (4) (2014) 351–359.
[168] M. Tsolaki, E. Karathanasi, I. Lazarou, K. Dovas, E. Verykouki, A. Karakostas, K. Georgiadis, A. Tsolaki, K. Adam, I. Kompatsiaris, Efficacy and safety of Crocus sativus L. in patients with mild cognitive impairment: one year single-blind randomized, with parallel groups, clinical trial, J. Alzheimer's Dis. 54 (1) (2016) 129–133.
[169] M.R. Khazdair, M.H. Boskabady, M. Hosseini, R. Rezaee, A.M. Tsatsakis, The effects of Crocus sativus (saffron) and its constituents on nervous system: a review, Avicenna J. Phytomed. 5 (5) (2015) 376.
[170] N. Pitsikas, The effect of Crocus sativus L. and its constituents on memory: basic studies and clinical applications, Evid. Based Complement. Altern. Med. 2015 (2015).
[171] P. Russo, A. Frustaci, A. Del Bufalo, M. Fini, A. Cesario, Multitarget drugs of plants origin acting on Alzheimer's disease, Curr. Med. Chem. 20 (13) (2013) 1686–1693.
[172] A. Nacu, R. Hoerr, Neuropsychiatric symptoms in dementia and the effects of Ginkgo biloba extract EGb 761® treatment: additional results from a 24-week randomized, placebo-controlled trial, Open Access J. Clin. Trials 8 (2016) 1.